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This research was an attempt to gain a better understanding of the processes involved in the microbial degradation of synthetic polymers. It involved the examination of extracellular and cell associated enzymes capable of cleaving the model polyester, polycaprolactone. An effort was made to compare these enzymes to gain some understanding of their multiplicity and activity. As information was gained regarding the degradation process we attempted the modification of other polymers to render them degradable or more degradable.

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The Mechanisms of Biodegradation of Synthetic Polymers

Final Report

J.A. Cameron and S. J. Huang

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3. Appendixes - None

4. A. Statement of the problem studied.

This research was an attempt to gain a better understanding of the processes involved in the microbial degradation of synthetic polymers. It involved the examination of extracellular and cell associated enzymes capable of cleaving the model polyester, polycaprolactone. An effort was made to compare these enzymes to gain some understanding of their multiplicity and activity. As information was gained regarding the degradation process we attempted the modification of other polymers to render them degradable or more degradable.

B. Summary of the most important results.

The majority of the work involved the yeast Cryptococcus laurentii, representing a single cell form of microbial degrader, and several members of the genus Fusarium, in particular Fusarium moniliforme, representing a filamentous organism involved in degradation. The extracellular enzyme of Cryptococcus laurentii was intensively studied. It has a high specific activity, a low molecular weight and is active over a relatively wide range of temperature and pH values. It is produced extracellularly in the late logarithmic stage of growth. The primary endproducts of its activity were trimers of caproic acid. While we were unable to demonstrate other proteins in our best extracellular enzyme preparations, we were unable to conclusively state that it was a pure preparation. The level of total protein was always very low when these organisms were grown in minimal medium, precluding assurance that some traces of other protein were not present following permeation chromatography. Even when the enzyme was concentrated by ultrafiltration, purified by gel permeation chromatography, and reconcentrated we could not verify purity because every method attempted led to a loss of enzyme activity. The enzyme apparently bound irreversibly to all of the ion exchange matrixes used as well as hydrophobic interaction media. When electrophoresis was attempted the enzyme activity was lost under all conditions. Earlier findings that a low molecular weight cofactor was necessary for enzyme activity were found to be spurious. We were unable to demonstrate any cell-associated enzyme by preparing protoplasts and looking for activity on the protoplast. The findings with Cryptococcus indicate that the organism is capable of elaborating significant amounts of hydrolytic enzyme which breaks the polymer down to a size that can be imbibed and utilized by the organism. This is the same pattern that we have seen in the bacterial genus Pseudomonas. Members of the genus Fusarium were found to vary considerably in their ability to degrade polycaprolactone but none of them elaborated large amounts of the enzyme, as judged by the size of the zone of clearing on polymer-agar plates. It was

found that Fusarium moniliforme, the most active organism on plates, produces only small amounts of enzyme extracellularly. When subjected to gel permeation chromatography three peaks of activity were seen. We are presently evaluating these peaks to determine whether the lower molecular weight ones are monomers of the largest one or whether they are independent enzymes. A preliminary attempt to electrophorese the preparations showed the same problem seen with the Cryptococcus enzyme, a loss of all activity. When protoplasts of Fusarium strains were prepared enzyme activity could be demonstrated in association with the protoplast. Holding the protoplasts in appropriate medium (proper salt and osmotic balance with nutrients to support metabolism) enzyme washed off the protoplasts and no more was produced, as they lost activity. Thus it appears that, in the organisms studied, two types of organisms are found, those that release relatively large amounts of efficient extracellular enzyme into their surroundings, and organisms that produce the enzyme in a periplasmic site, releasing relatively small amounts of it and retaining significant amounts in association with the cell.

We have also examined the effects of the modification of polymers on their biodegradability. The effect the catalyst used for polymer synthesis was examined for determination of their role in resistance or susceptibility to biodegradation. Para-toluene, tin chloride and zinc chloride were used as catalysts in the preparation of poly(hexamthylene tartrate) and poly(octamethylene tartrate) and their polyurethane derivatives. Para-toluene and tin chloride resulted in polymers that were susceptible to degradation by Aspergillus niger. On the other hand, the use of zinc chloride led to an inhibition of fungal degradation. This was apparently due to the release of zinc, which is known to be fungicidal and fungistatic, which had been entrapped in the polymer, and its action on the fungi as it was released into solution. The release was found to be linear with the square root of time. The effect of surface modification of aromatic polymers on their degradability was examined by treating polystyrene and poly(ethylenepterephthalate) (PET) with strong oxidizing agents. The polystyrene showed very little change but the PET showed marked surface changes, yielding a rough, irregular surface. These polymers did not, however, support growth of Fusarium moniliforme or other fungi any better than the original polymer. Heat treatment (120° C) and treatment with polyester depolymerase from Cryptococcus laurentii resulted in further surface changes, leading to the ability to support the growth of Fusarium. Enzyme treated surfaces were even more rough than untreated surfaces. Heated surfaces showed dramatic changes with a rough and convoluted form.

C. Publications

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C. Participating personnel.

J. A. Cameron, S. J. Huang, Laura J. DiBenedetto (PhD. Dec. 1989), Albert S. Costa (M. S. Dec. 1986), Lida Kimmel (M.S. 1989), Paul Urbanowski (M.S. 1988), Elizabeth Geiger.

5. Bibliography - None

6. Appendixes - None